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REVIEW

Neuroimmune mechanisms in functional bowel disorders

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ABSTRACT

The enteric nervous system regulates diverse functions including gastrointestinal motility and nociception. The sensory neurons detect mechanical and chemical stimuli while motor neurons control peristalsis and secretion.
In addition to this extensive neuronal network, the gut also houses a highly specialised immune system which plays an important role in the induction and maintenance of tolerance to food and other luminal antigens and in the protection of the epithelial barrier against pathogenic invasion.
It is now increasingly recognised that the gastrointestinal immune system and the enteric nervous system closely interact. This review will focus on two common functional gastrointestinal disorders in which neuroimmune interaction is involved in the pathophysiology: i.e. postoperative ileus and irritable bowel syndrome. Postoperative ileus arises after almost every abdominal surgical procedure. Handling of the bowel results in local inflammation and activation of inhibitory neuronal pathways resulting in a generalised impairment of gastrointestinal motor function or ileus. On the other hand, postinfectious irritable bowel syndrome (PI-IBS) occurs in 10 to 30% of patients who suffer from infectious gastroenteritis. PI-IBS patients develop abnormal gastrointestinal sensitivity, motility and secretion which contribute to abdominal pain and discomfort, bloating and abnormal bowel function (diarrhoea and/or constipation). Biopsy studies revealed persistent low-grade inflammation and altered immunological function which may lead to abnormal pain perception and motor activity within the gastrointestinal tract.

KEYWORDS

Post operative ileus, post infectious irritable bowel syndrome, macrophage, mast cell

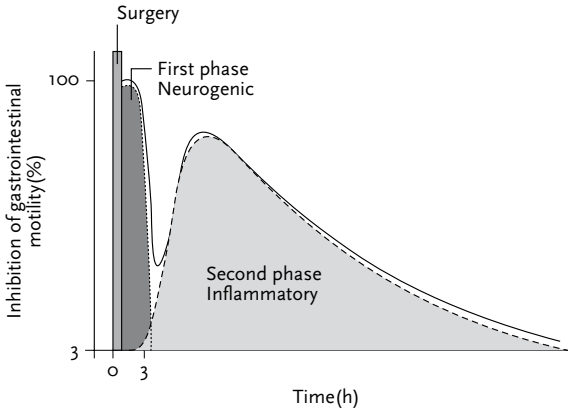
INTRODUCTION

It is generally accepted that there is bi-directional communication between the central nervous system (CNS) and the enteric nervous system (ENS). Indeed, the brain is continuously informed by afferent nerves detecting gut activity, whereas it is well established that psychological state or stress has a major influence on gut function. Interestingly, recent evidence shows that this bi-directional communication along the brain-gut axis is not confined to gut digestion and motility, but also involves immunological mechanisms, i.e. the immune system affects neuromuscular function whereas the nervous system has a major modulatory input on the immune system. In this review, we present evidence to support this bi-directional communication in two gastrointestinal disease models, postoperative ileus and postinfectious irritable bowel syndrome.

PATHOPHYSIOLOGY OF POSTOPERATIVE ILEUS

Postoperative ileus (POI) occurs after every abdominal surgical procedure. It presents clinically as the inability to tolerate food, the absence of bowel sounds, lack of flatus and defecation and abdominal distention. On average, this period lasts two to four days for conventional abdominal procedures.
By now, it is well established that POI is caused by transient inhibition of gastrointestinal motility which involves the entire gastrointestinal tract. Intestinal handling triggers two different phases, each with its own dynamics and underlying pathophysiological mechanism (*figure 1*). The first or early phase is neurally mediated and involves neural reflexes activated during and immediately following surgery. In a later phase, leucocytes enter the manipulated

Figure 1. *Schematic representation of the two phases involved in postoperative ileus*



The first neurogenic phase starts during abdominal surgery and ends soon after it. The second inflammatory phase starts approximately three to four hours after surgery, lasts much longer and is therefore clinically more relevant. (Figure adapted from Gut 2009; 58:1300-11).

intestinal segments, impairing the contractile properties of the inflamed intestine. This second phase starts three to four hours after surgery and is triggered by activation of resident macrophages in the muscularis externa. This later phase is responsible for the sustained and thus clinically more relevant inhibition of gastrointestinal motility.

THE LATE INFLAMMATORY PHASE OF POI

The second, long-lasting phase of POI is mainly due to inflammation of the intestinal muscularis.[1,2] Mainly from animal studies, it is now generally accepted that inflammation of the intestinal muscularis is the key mechanism impairing gastrointestinal motility resulting in postoperative ileus. Also in humans, we recently demonstrated that intestinal manipulation yields influx of neutrophils into the manipulated intestinal loops. Most evidence so far has identified mast cells, most probably peritoneal mast cells, and resident macrophages as the main players of the innate immune system involved in the inflammatory response to intestinal handling.

Resident peritoneal mast cells are a minor population of differentiated cells in the peritoneal cavity. Activation of these peritoneal mast cells by intestinal manipulation and the subsequent release of mediators such as histamine and mMCP-1 (murine mast cell protease 1) was demonstrated in rodent models and even in human.[3,4] Tryptase, one of the typical mediators released by mast cells, was significantly increased in the peritoneal lavage collected during abdominal surgery by gentle inspection of the intestine. In an animal model, mast cell stabilisers such as ketotifen and doxantrazole reduced the inflammatory response and improved gastric emptying 24 hours after abdominal surgery.[5,6] Moreover, transgenic mice that lack mast cells failed to develop an intestinal infiltrate following intestinal manipulation while reconstitution of these mast cells restored the capacity of mutant animals to recruit leucocytes to the intestine after surgery.[6] These data clearly support a key role for peritoneal mast cell activation in the development of POI but to date, the exact triggers activating these cells are still unclear.

Although tissue damage following intestinal handling will certainly contribute to the inflammatory cascade, mast cell activation is considered the most important step (*figure 2*). The mediators released by mast cells increase intestinal permeability, facilitating translocation of intraluminal bacteria and bacterial products (*figure 2*). In animal studies, introduction of fluorescent lipopolysaccharide or fluorescent microbeads into the intestines prior to surgery results in translocation of this fluorescent material through the mucosa into the intestinal wall. Once the beads enter the intestinal wall, they were transported to the lymph nodes via the lymphatic system or phagocytosed by the resident macrophages.[7] These data confirm that bacterial translocation occurs in response to surgery and may contribute to the activation of the immune system, in particular resident macrophages.

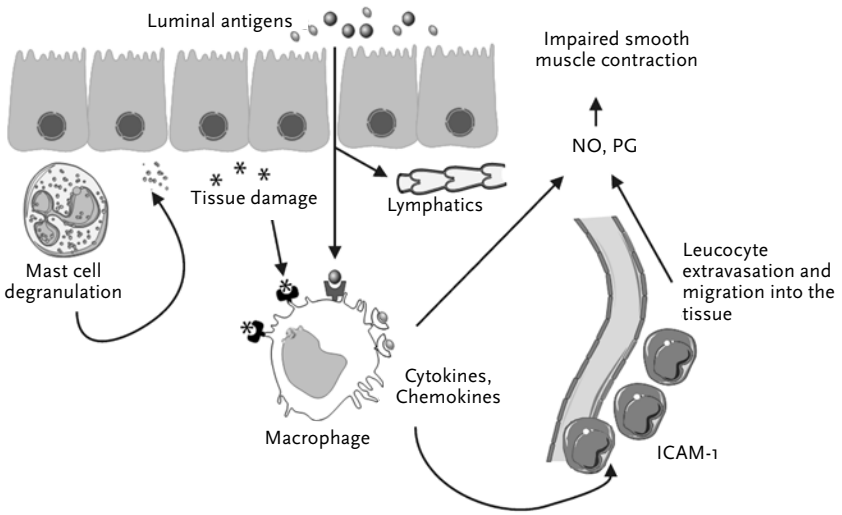
Residential macrophages are normally quiescent macrophages organised into a layer or 'network' at the level of the myenteric plexus and at the serosal side of the intestine. It has been suggested that these macrophages become activated by translocated bacterial antigens but also by the degradation products of extracellular matrix released during surgical manipulation (*figure 2*). Animal studies showed that pharmacological or genetic depletion of resident macrophages decreased the release of inflammatory mediators and diminished the recruitment of leucocytes in the muscularis.[8] In addition, macrophage-altered animals had near normal *in vitro* jejunal circular muscle function and gastrointestinal transit despite surgical manipulation, clearly illustrating the importance of these phagocytes in POI.

Stimulated macrophages secrete proinflammatory cytokines and chemokines leading to the upregulation of adhesion molecules (ICAM1) in the endothelium and the progressive influx of leucocytes (*figure 2*). Leucocytes and activated resident macrophages will synthesise enzymes such as iNOS and COX-2 which contribute to the impaired gastrointestinal motility, the main characteristic of POI.

NEUROIMMUNE INTERACTIONS IN POI

Proinflammatory cytokine production by innate immune cells and their release in the blood stream is critically associated with the pathology of inflammatory disorders

Figure 2. *Proposed immune mechanisms underlying impaired intestinal contractility following abdominal surgery*



Mast cells are activated by intestinal handling and release substances which increase mucosal permeability. This allows luminal bacteria or bacterial products such as lipopolysaccharides to enter the lymphatic system or to interact with Toll-like receptors on residential macrophages. The degrade products of the extracellular matrix that are released upon tissue damage by intestinal manipulation may also directly activate residential macrophages. The latter will release inflammatory cytokines and chemokines, resulting in upregulation of endothelial adhesion molecules including intercellular adhesion molecule-1 (ICAM-1). The latter attracts invasion of leucocytes into the intestinal muscularis externa. These leucocytes and the resident macrophages concomitantly produce large amounts of nitric oxide (NO) and cyclo-oxygenase 2 (Cox-2). Cox-2 stimulation results in production of prostaglandins (PG) which together with NO, impair the contractile activity of the smooth muscle cells.

including POI. Recently it became clear that the brain can downregulate this inflammatory response through a parasympathetic anti-inflammatory pathway.

The first studies on the role of the central nervous system in regulating systemic inflammatory responses were performed by Tracey *et al*. They demonstrated that activation of afferent vagus nerve fibres by inflammatory endotoxins or cytokines stimulated a parasympathetic anti-inflammatory pathway. Acetylcholine, the principle vagal neurotransmitter, significantly attenuated the release of proinflammatory cytokines[9] while direct electrical stimulation of the peripheral vagus nerve during lethal endotoxaemia prevented the development of shock.[9] Since then, the anti-inflammatory effect of vagal nerve stimulation has been demonstrated in models of pancreatitis,[10] ischaemia[11] and colitis.[12] Also in a mouse model of POI, De Jonge *et al*. showed that the vagus nerve exerts an anti-inflammatory action *via* activation of the alpha7 subunit of the nicotinic receptor.[3]

THERAPY

Various reports on animal research confirmed the modulatory role of the cholinergic anti-inflammatory pathway on the intestinal immune system,[3,12] whereas vagotomy ameliorated inflammation. These reports open new perspectives in the development of new anti-inflammatory compounds. Agonists that mimic the effect of the vagal nerve can be developed to treat POI. We previously showed that pretreatment with a specific alpha7 nicotinic agonist indeed prevents inflammation and postoperative ileus in mice.[13] An alternative approach is to activate the endogenous vagal anti-inflammatory system. This can be achieved by feeding a high fat diet. Dietary fat induces the release of cholecystokinin which activates the vagal nerve indeed resulting in an anti-inflammatory effect[14] in animal models of sepsis and POI.[14] Maybe this clarifies the protective effect of fast-track surgery against POI.[15-17] Fast-track surgery promotes postoperative early ambulation and early oral hydration and nutrition.

Finally, as mast cells are playing an important role in the initiation of the inflammatory cascade triggered by intestinal handling, and mast cell stabilisation has proven efficient in our murine model, we designed a pilot study evaluating the effect of the mast cell stabiliser ketotifen on postoperative ileus in patients undergoing major abdominal surgery for gynaecological malignancy.[5] In this study, mast cell stabilisation restored gastric emptying and relieved abdominal cramping.[5]

In summary, intestinal inflammation due to handling of the intestines activates mast cells and resident macrophages which results in an influx of leucocytes and an inflammatory response leading to impaired motility and ileus. Minimising intestinal handling and fast track surgery shorten POI while mast cell stabilisation and drugs or interventions mimicking the effect of the vagal anti-inflammatory pathway may represent new approaches for the treatment of POI.

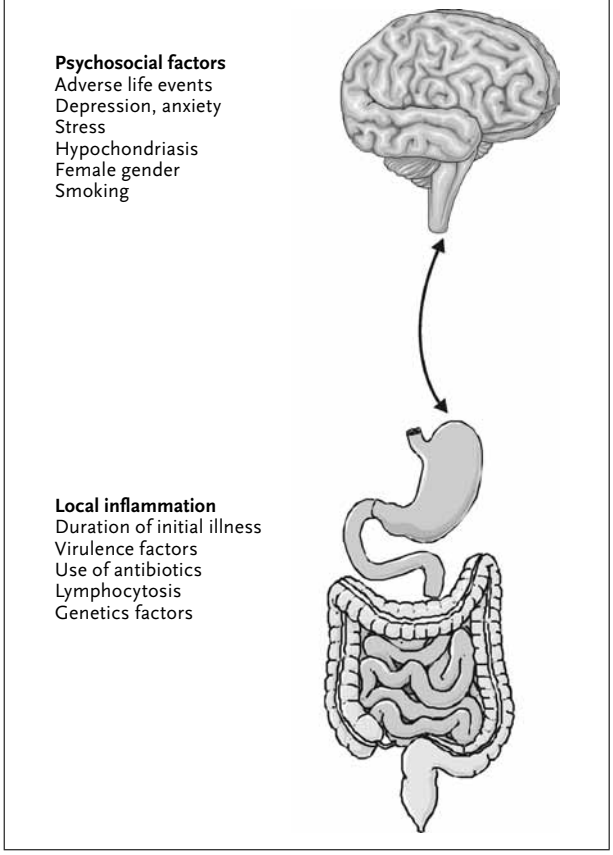
PATHOPHYSIOLOGY OF POSTINFECTIOUS IRRITABLE BOWEL SYNDROME

The irritable bowel syndrome or IBS is the most common gastrointestinal disorder affecting approximately 15% of individuals worldwide. IBS is characterised by chronic abdominal pain, discomfort, bloating, and alteration of bowel habits in the absence of any detectable organic cause. Increased abdominal pain perception or visceral hypersensitivity is considered an important pathophysiological mechanism explaining the clinical presentation of IBS. The diagnosis is based on IBS symptoms as described in Rome III criteria as no clinical tests nor imaging techniques can positively identify IBS.

In some individuals, IBS has an acute onset following an infectious gastroenteritis. This postinfectious syndrome has consequently been termed 'postinfectious IBS' (PI-IBS). Published studies have reported the incidence of PI-IBS after an infection ranges between 5% and 32%. Various bacterial pathogens including *Campylobacter, Shigella, Salmonella* and *Escherichia coli* have been implicated in the development of PI-IBS. Whether all organisms confer an equivalent risk remains unclear.

Potential risk factors for developing long-standing gastrointestinal symptoms after an acute infection include female gender and environmental factors (smoking, psychological distress) (*figure 3*). Especially a recent traumatic life event or a neurotic personality trait increase the susceptibility to develop PI-IBS. Genetic risk factors that underlie the susceptibility to develop postinfectious IBS include gene alterations in TLR9, a gene responsible for bacterial recognition, IL6, involved in inflammatory response and cadherin-1 (CDH1), involved in epithelial integrity.[18] In addition, factors related to the pathogen or the infection including the duration and severity increase the risk of developing postinfectious IBS (*figure 3*). Based on these risk factors it appears that the interaction between local inflammatory factors and psychosocial factors plays an important role in the development of PI-IBS, which fits well with the current theory of brain-gut dysfunction in patients with IBS. The exact mechanisms behind the development of long-standing gastrointestinal symptoms after an infectious event are not clear.

Figure 3. *Interaction between local inflammation and psychosocial factors in determining the risk of developing postinfectious IBS*



INTESTINAL BARRIER FUNCTION

Compromised epithelial barrier function has been associated with both IBS and PI-IBS.[19,20] Marshall *et al.* demonstrated a high incidence of postinfectious IBS with increased permeability following an acute bacterial gastroenteritis after a waterborne outbreak. This aberrant barrier function in IBS patients is due to decreased expression of tight junction proteins including zona occludens protein 1 (ZO-1).[21] It remains unclear, however, why these proteins are affected in IBS patients. Interestingly, increased permeability was evoked in mice when faecal supernatants from patients with diarrhoea-predominant IBS were applied to the colonic mucosa,[22] clearly indicating the presence of certain components that affect epithelial barrier function. It has been speculated that proteases such as tryptase (endogenous or deriving from commensal bacteria) may play an important role. Recent studies demonstrated the role of protease-activated receptors (PAR) in barrier dysfunction and in the generation of IBS symptoms. Activation of PAR1 and 2 on enteric neurons provokes the release of neuropeptides[23] which in turn activate their receptors present on endothelium and mast cells. Such PAR-induced micro-inflammation might participate in the generation of IBS symptoms as low levels of inflammation have been proposed to be involved in the pathogenesis of hypersensitivity. PAR2-induced permeability and rectal hypersensitivity could be inhibited by a tight junction blocker.[24] These studies demonstrate an important role for increased intestinal permeability in the development of PI-IBS hypersensitivity.

NEUROIMMUNE INTERACTIONS IN PI-IBS

Mast cell activation

As stated above, increased abdominal pain perception or visceral hypersensitivity is the hallmark of IBS. A variety of animal models of visceral hypersensitivity demonstrated immune activation with subsequent microscopic inflammation as the underlying mechanism of abnormal pain perception. Especially the role of mast cell activation in postinfectious IBS has been extensively studied. In PI-IBS animal models using *Trichinella spiralis*[25-27] or *Nippostrongylus brasiliensis*[28,29] infections, the development of visceral hypersensitivity has been associated with increased mast cell numbers and/or activation. Similarly, several studies indicated an increase in mast cell numbers in close proximity of nerve fibres in intestinal biopsies of IBS patients[30] demonstrating interactions between mast cells and nerves may be relevant for symptom generation. In addition, the supernatant of patient biopsies which contains more mast cell mediators[31] evoked an increased *in vivo* pain response to colonic distension in mice.[32] The importance of mast cell mediators was also addressed in a study by Buhner *et al*., who reported that the supernatants of colonic biopsy samples from IBS patients but not of those from healthy controls, activate human submucosal neurons.[33] Interestingly, this activation was not associated with IBS subtype (constipation/diarrhoea/alternating IBS), which indicates that it might be a general pathogenetic mechanism of IBS. In another study, peripheral blood mononuclear cell supernatants from patients with postinfectious IBS, but not of controls, activated mouse colonic pelvic or lumbar splanchnic nerves, which further suggests that the immune system is potentially involved in the generation of visceral hypersensitivity in IBS.[34] Taken together, mast cells release neurally active mediators (histamine, proteases, prostaglandins) which sensitise afferent neurons inducing increased visceral pain perception. On the other hand, mast cells also express a variety of neuropeptide receptors, demonstrating the significance of mast cells as end effector cells of the brain gut axis in the intestinal mucosa.

Adaptive immunity

It has been hypothesised that an adaptive immune response may underlie visceral hypersensitivity. Serial rectal biopsies taken from patients who developed IBS after a *Campylobacter jejuni* gastroenteritis showed a persistent inflammatory infiltrate, with an increase in enterochromaffin cells, mast cells and lymphocytes.[20] The recent finding that antibodies against nonspecific bacterial products, flagellin, are increased in IBS patients[35] together with the increase in IgG+ B cells in PI-IBS patients[36] underscores the importance of an adaptive immune response in PI-IBS. Therefore, it is hypothesised that PI-IBS patients develop a chronic low-grade immune response against commensal microbiota. Although these studies indeed indicate a role for immune activation in PI-IBS, there are some discrepancies in the literature that remain to be further studied. Two studies reported increased numbers of T lymphocytes[20,37] in PI-IBS whereas another study described normal T cell numbers in intestinal tissue.[38] Also the cytokine profile of mucosal T cells of (PI-) IBS patients has not been studied yet. More detailed studies of T and B cell activity and altered antibody production in IBS will most certainly result in a better understanding of IBS symptom generation.

THERAPY

Although the mechanisms by which initial inflammation triggers a state of visceral hypersensitivity remain unclear, it is thought to be related to modulation of visceral sensory neurons by mast cell mediators. More evidence of mast cell involvement in IBS is provided by drug studies such as a study by Klooker *et al*. In this study, treatment of IBS patients with the mast cell stabiliser ketotifen resulted in decreased visceral hypersensitivity and improved intestinal symptoms.[39] Another small study reported reduction in mast cell numbers and mediators following treatment with the anti-inflammatory drug mesalazine.[40] In mice, early corticosteroid therapy has been shown to attenuate postinfectious neuromuscular dysfunction.[41] However, a small randomised controlled trial with 29 PI-IBS patients given 30 mg prednisolone/day for three weeks was negative.[42] As stated above, disruption of intestinal barrier integrity plays an important role in PI-IBS and improving its function and integrity by glutamine supplementation may represent a new therapeutic approach.[43] Also therapeutic alteration of the GI microbiota by probiotic bacteria was shown to improve IBS symptoms and restore intestinal homeostasis.[44]

In summary, PI-IBS is triggered by an acute gastroenteritis and is characterised by increased mucosal permeability and a chronic low-grade inflammatory response in the mucosa. Mast cell activation plays a crucial role and their mediators sensitise nociceptive nerve fibres, thereby inducing increased abdominal pain perception. There is no standard treatment available so far but pilot studies indicate the beneficial role of mast cell stabilisers, anti-inflammatory drugs, glutamine supplementation and probiotics.

CONCLUSION

In conclusion, postoperative ileus and postinfectious IBS are the result of abnormal neuroimmune interactions. Inflammation in POI involves mast cell and subsequent

macrophage activation followed by an influx of leucocytes and impaired motor function. The vagal nerve exerts a cholinergic anti-inflammatory pathway and its activation may represent a new therapeutic approach in the treatment of POI. In contrast, inflammation in PI-IBS involves chronic loss of epithelial barrier integrity followed by mast cell activation and chronic low-grade inflammation. Release of proinflammatory mast cell mediators sensitise nociceptive neurons resulting in increased visceral pain sensitivity. Future research on the nature of the inflammatory response will provide insight and new tools to prevent and treat POI and PI-IBS.

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